A Review on DNA methylation profiling, a research article with related Commentary on acute myeloid leukemia (AML), and a research article concerning myotonic dystrophy type I (DM1) were selected for the January 2013 JMD CME Program in Molecular Diagnostics. The authors of the referenced articles and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.


Upon completion of this month’s journal-based CME activity you will be able to:

- Understand the biochemistry of DNA methylation and the role it plays in transcriptional regulation.
- Describe the diagnostic implications of DNA-methylation of genes.
- Describe the advantages and disadvantages of several different techniques used to detect gene methylation.
- Describe the incidence of AML.
- Discuss the laboratory techniques that are used to diagnose AML.
- Describe the fms-related tyrosine kinase 3 gene (FLT3) and its importance in AML.
- Describe the molecular pathophysiology of DM1.
- Discuss the advantages and disadvantages of Southern blot analysis and amplification methods in the diagnosis and prognosis of DM1.

1. DNA methylation, an epigenetic alteration, plays a key role in transcriptional control. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. DNA methylation occurs when a methyl group is added to the fifth carbon of adenine or to the sixth carbon of cytosine.

b. Aberrant cytosine methylation is associated with silencing of tumor suppressor genes.

c. Alterations in the methylation status of DNA are promising candidates for a highly specific and sensitive indicator of cancer diagnosis and prognosis.

d. DNA methylation is crucial for a variety of processes, such as genomic imprinting, X-chromosome inactivation, and suppression of repetitive elements.
2. Changes in DNA methylation in cancer include both global hypomethylation and gene-specific hypermethylation. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Genome-wide hypomethylation results in chromosomal instability and increased mutation rates.
b. Promoter hypermethylation suppresses gene transcription either by preventing transcription factors from binding to the gene or by altering chromatin structure.
c. DNA hypomethylation contributes to oncogenesis by point mutation and inactivation of tumor suppressor genes whereas hypermethylation may lead to chromosomal instability and activation of proto-oncogenes.
d. Tumor growth is characterized by genome-wide hypomethylation, accompanied by hypermethylation of tumor suppressor gene promoters caused by increased expression of DNA methyltransferases.

3. CpG methylation analysis is useful in assessing tumor progression, disease classification, diagnosis, and prognosis of various types of human cancer. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. In breast cancer diagnosis, CpG abnormality of genes, such as ESR1, CDKN2A, PAR, MDGI, CALCA, CDH1, LATS1, and LATS2, is well illustrated.
b. CpG methylation is useful in monitoring the progression of cervical cancer.
c. Promoter-specific CpG methylation aberration is relatively higher in DAPK1, CADM1, and CDKN2A genes in invasive cervical cancers than in high-grade squamous intraepithelial lesions.
d. Methylation analysis cannot be used for risk assessment of patients with prolonged viral and bacterial infections.

4. Blotting is a conventional technique for DNA methylation analysis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Antibodies raised against 5-methylcytosine are applied to a DNA sample immobilized on a nitrocellulose membrane to identify 5-methylcytosine in DNA.
b. The 5-methylcytosine sequentially reacts with primary antibody and radiolabeled secondary antibody and is then visualized using autoradiography.
c. The complication of partial renaturation of double-stranded DNA blotted on the nitrocellulose membrane can be eliminated by using reversible denaturation reagents.
d. In concentrated DNA samples, the efficiency of detection is diminished due to steric hindrance caused by the two Fab (antigen-binding fragment) arms of the antibody, which can be avoided by using an Fab instead of the whole antibody.

5. Conventional sequencing methods use Maxam and Gilbert chemical cleavage reactions, along with amplification procedures, to establish the methylation status of the promoters in tumor-responsive genes. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Conventional sequencing methods can detect protein-binding sites on genomic DNA in vivo.
b. It is easy to identify 5-methylcytosine residues using conventional Maxam and Gilbert chemical cleavage reactions.
c. Maxam and Gilbert sequencing protocols cannot be used for small or mixed DNA samples.
d. Bisulfite sequencing is widely used for mapping of promoter hypermethylation and epigenotyping.

6. Methylation status can be assessed on DNA fragments cleaved by methylation-sensitive restriction enzymes using Southern blot hybridization techniques or PCR amplification. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Longer fragments that result from the inability of the enzymes to cleave methylated sequences indicate a methylated CpG dinucleotide.
b. Restriction enzyme-based methods are simple, rapid, and extremely sensitive.
c. Restriction enzyme-based methods are limited to specific restriction sites and require a substantial amount of high-quality DNA.
d. Restriction enzyme-based methods cannot be used for genome-wide methylation analysis and marker discovery techniques.

7. In the United States, acute myeloid leukemia (AML) develops in approximately 13,000 individuals annually and accounts for approximately 9,000 cancer-related deaths per year. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

a. Epidemiologically AML is considered a single disease, but it is not a uniform clinicopathologic entity.
b. Some patients respond well to chemotherapeutic regimens, others require hematopoietic stem cell transplants, and yet others rapidly die of AML.
c. The survival of patients with AML is independent of age and performance status.
d. Molecular characteristics of tumor cells are the major factors that influence prognosis of AML.
8. The first recognized and best-studied recurrent genetic lesions in AML are large chromosomal anomalies. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

   a. The large chromosomal abnormalities are usually detected by metaphase cytogenetic analysis.
   b. Translocations and inversions such as t(8;21), inv(16), and t(15;17) confer a relatively good prognosis.
   c. Chromosomal gains and losses such as monosomy 7 confer a poor prognosis.
   d. All AML cases can be recognized by either traditional karyotyping or by fluorescence in situ hybridization (FISH).

9. The fms-related tyrosine kinase 3 gene (FLT3) encodes a class III receptor tyrosine kinase that is required for normal hematopoiesis. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

   a. Recurrent mutations in FLT3 have been found in approximately 50% of AML cases overall.
   b. Recurrent FLT3 mutations fall into two broad categories: internal tandem duplications (ITDs) within the juxtamembrane domain and point mutations within the kinase domain.
   c. Both ITDs within the juxtamembrane domain and point mutations within the kinase domain of mutations render the kinase constitutively active.
   d. Only the ITD mutation within the juxtamembrane domain has been definitively shown to correlate with prognosis.

10. Recent genomic studies have demonstrated that besides FLT3 mutations, recurrent mutations in several other genes may be informative prognostic markers in AML. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

    a. Mutations in NPM1, CEBPA, WT1, KIT, DNMT3A, IDH1, IDH2, TET2, and ASXL1 have all been found to occur in a significant fraction of AML patients.
    b. Mutations in RUNX1, MLL, and NRAS are not associated with AML.
    c. Focal testing of exons and known hotspots for mutations in some genes is currently available.
    d. Next-generation sequencing (NGS)-based approaches are theoretically capable of identifying all types of mutations observed in AML.

11. Myotonic dystrophy type 1 (DM1) is an autosomal-dominant disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:110-115.]

    a. DM1 is caused by an expansion of CTG repeats in the 3' untranslated region of the dystrophia myotonica protein kinase gene (DMPK).
    b. Unaffected individuals carry two alleles of 5 to 34 CTG-repeat units.
    c. DM1 patients carry an expanded mutant allele of more than 50 CTG repeats, sometimes more than 2,000 repeat units.
    d. The disease severity is independent of the number of CTG repeats.

12. Southern blot analysis of enzymatically digested genomic DNA is a widely used method for molecular diagnostics of CTG expansions in DM1. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:110-115.]

    a. Southern blot analysis is time consuming, low through-put, and requires large amounts of good-quality genomic DNA.
    b. PCR-based methods represent a promising strategy for DM1 detection.
    c. A detection limit of ~80 to 100 CTG repeats is common in conventional PCR methods used for molecular diagnostics of DM1.
    d. Expansions of ~12,000 CTG repeats have been detected after direct visualization of PCR products on agarose gels using various modifications of PCR reagents and enzymes.